REMARKS

The Examiner has not found the traversal of the restriction requirement to be persuasive. The Examiner has deemed the restriction requirement proper.

Claims 1 – 38 are currently pending in the application. Claims 22 – 38 have been withdrawn from consideration. Claim 13 has been objected to and claims 1 – 21 have been rejected. Claims 4 and 13 have been amended. No new matter has been added.

Claims 4 and 13 have been objected to for containing informalities. Claims 4 and 13 have been amended to correct for the typographical errors.

The specification was objected to for containing an embedded hyperlink, and for use of uncapitalized trademark(s). Applicants have made the appropriate corrections. Support for the amendments can be found in the specification and claims as originally filed.

Accordingly, Applicants respectfully request withdrawal of the objections.

Claim Rejections- 35 U.S.C. § 112, first paragraph

Claims 1 – 21 have been rejected under 35 U.S.C. §112 for failing to comply with the written description requirement. The Examiner argues "the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention." Applicants respectfully traverse the rejection.

The claims of the instant application encompass nucleic acids comprising, consisting essentially of, and having SEQ ID Nos: 1 and 2 (corresponding to Probe A and Probe B, respectively). The Examiner argues that the specification has only described nucleic acids

consisting of SEQ ID No: 1, and the nucleic acids consisting of SEQ ID No: 2. The Examiner argues, "the claims encompass a large genus of sequences (and)... (t)he specification does not provide guidance as to what constitutes SEQ ID No: 1 and 2 (page 21, Office Action)." Thus, the Examiner contents that "(t)he nucleic acids comprising, consisting essentially, and hav(ing) SEQ ID No: 1 and 2 are not synonymous with microbial resistant bacteria (pages 21-22, Office Action)." The Examiner alleges that examples in the art teach "sequences comprising, consisting essentially, and have SEQ ID No: 1 and 2 that have different functions and are isolated from a variety of species (page 22, office Action)." Thus, the Examiner concludes that the specification fails to provide an adequate description of a representative number of species from the genus. Applicants disagree with the Examiner's rejection.

The claims in the instant Application are directed to a method for the analysis of a target sequence in a sample, the method comprising contacting the sample with at least one mixture of two probes, Probe A (SEQ ID. No. 1) and Probe B (SEQ. ID No. 2).

The specification has provided ample description for one skilled in the art to determine what the nucleic acids comprising SEQ ID No: 1, and the nucleic acids comprising SEQ ID No: 2 could be. On page 5, the specification first teaches that the probes are defined by their hybridization to a target region of DNA or RNA:

> Accordingly, and in one aspect, the invention features a method to analyze a target sequence in a sample. In one embodiment, the method includes contacting the sample with at least one mixture of two probes (called Probe A and Probe B for convenience). In a more specific embodiment, the two probes are characterized as follows: i) Probe A is comprised of a nucleotide sequence, which hybridizes to a target region of both wanted and unwanted DNA or RNA and is labeled with a fluorophore at the end which, upon hybridization is closest to Probe B; and ii) Probe B is comprised of a nucleotide sequence which hybridizes to the target region of unwanted DNA or RNA adjacent to the target region of Probe A and is labeled with a quencher at the end which, upon hybridization is closest to Probe A. Preferably, the method further

> includes detecting, identifying or quantitating the hybridization of Probe A to the target sequence, under suitable hybridization conditions, wherein the presence or amount of wanted DNA or RNA present in the sample can be positively correlated with the fluorescence of the fluorophore of Probe A.

The specification has defined the probes and their function by their hybridization characteristics. Accordingly, the specification defines the hybridization characteristics of a probe (i.e. Probe A (SEQ ID No: 1) and Probe B, (SEQ ID No: 2) and teaches in detail the parameters of the hybridization characteristics of the probes. Applicant directs the Examiner to page 15 of the specification:

> The hybridization characteristics of a probe are usually described by the melting point (Tm) of the probe-target hybrid. The melting point is therefore an important parameter used to guide the experimentation described above to determine the suitable hybridization conditions. However, when the assay is dependent on simultaneous hybridization of two probes each of these two probes must to be designed with similar hybridization characteristics such that the same hybridization conditions are suitable for both probes. The length of the nucleobase sequence provides a rough assessment of the hybridization characteristics, but can be refined by calculating the Tm using on-line calculators available at www.appliedbiosystems.com. The degree of similarity between the hybridization characteristics of Probe A and Probe B is dependent on both the stringency of the hybridization conditions and the desired degree of discrimination that needs to be achieved. Aided by no more than routine experimentation and the disclosure provided herein, those of skill in the art will easily be able to determine the degree of similarity required for performing assays utilizing the methods and compositions described herein.

Further, Figure 1A teaches specifically hybridization of the Probe A to the target sequence of *Staphylococcus aureus* and of the Probe B nucleobase sequence to the non-target sequence of *Staphylococcus schleiferi*. Of note is that even though there is a one base mismatch between Probe A probe and the *Staphylococcus schleiferi* target sequence, a relatively stabile hybrid is formed. The specification teaches that this stability is due in part to the total length of the probe (15 bases), and to the relatively high stability of G-T pairs (mismatches) as compared to all other PNA-NA mismatch

hybrids. Taken together, the specification provides ample teaching to one skilled in the art that the inventors were in possession of SEQ ID Nos: 1 and 2, (Probes A and B, respectively), at the time the application was filed. The specification teaches that hybridization defines the function of the sequences comprising, consisting essentially, and having SEQ ID Nos: 1 and 2.

Page 18 of the specification provides support for sequences comprising, consisting essentially, and having SEQ ID Nos: 1 and 2, and associates these sequences with function (i.e. hybridization):

> "For example, the Probe A can include the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and/or the Probe B can include the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA. In this embodiment, the probes can include other nucleobases (e.g., less than about 10, preferably less than about 5, usually one or two nucleobases), provided such additions do not detectably impact function of the probes.
>
> "However, in embodiments in which hybridization specificity is especially important, Probe A can consist essentially of the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and the first fluorophore and/or the Probe B can consist essentially of the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA and the quencher.
>
> "Where even more potential hybridization specificity is desired, the Probe A can consist of the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and the fluorophore. Also, the Probe B can consist of the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA and the quencher. In this embodiment, the Probe A is labeled with the first fluorophore at the probe terminus closest to the binding site of Probe B, and Probe B is labeled with a quencher at the probe terminus closest to the binding site of Probe A."

Applicants respectfully request the withdrawal of the rejections and allowance of the claims.

Claim Rejections- 35 U.S.C. § 112, second paragraph

Claims 1 – 21 have been rejected under 35 U.S.C. §112 for lacking clarity. The Examiner alleges "the claims are indefinite over the recitation 'from about'." The Examiner argues that "from

about" is not an art recognized term to describe the distance between two hybridized probes. Applicants respectfully traverse the rejection.

The specification provides ample description of hybridization conditions. See, for example, page 15 as referenced above. See also Figure 1 which teaches a specific example of hybridization using the two probes. The specification teaches one skilled in the art the parameters necessary for hybridization, thus one of skill in the art would be able to determine what the appropriate distance between the two probes would be. The specification teaches that the distance from about one to about five nucleobases is suitable for FRET analysis:

> In embodiments in which Probe A and Probe B are LNA or PNA probes such probes will be desirably separated by a distance of between from about one to about five nucleobases bases. In this invention example, FRET analysis is supported by the relatively close potential spacing between the fluorophore on Probe A and quencher on Probe B after hybridization to target.

Applicants respectfully request the withdrawal of the rejections and allowance of the claims.

Claim Rejections- 35 U.S.C. § 103(a)

Claims 1, 12, 13, and 21 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Heller (US 5,532,129, referred to herein as the '129 patent), in view of Elsas et al (US 6,207,387, referred to herein as the '387 patent). Applicants respectfully traverse the rejection.

The Examiner alleges that the '129 Patent teaches "a method to detect target sequences that comprises step a) hybridizing two probes, wherein one probe has a fluorophore (Probe A) and another probe that has a quencher (Probe B) ...and method step b) the subsequent detection of Probe A's flourescence as an indication of the presence of a target sequence (page 5, Office Action)." The Examiner admits, however, that the '129 patent does not teach a method wherein the

probes have wanted and unwanted sequences. The Examiner argues that the '387 patent cures the flaws of the '129 patent and teaches "of anchor and detection probes that detect mutations (page 6, Office Action)." The Examiner further alleges that Probe A of the '329 patent is the detection probe because it has wanted and unwanted sequences, and Probe B is represented by the anchor probe. The Examiner argues that it would have been prima facie obvious to modify the teachings of the '129 patent with the improvement of designing probes with wanted and unwanted sequences as taught by the '387 patent.

Neither the '129 patent nor the '387 patent, alone or in combination, teaches or suggests "wherein the presence or amount of wanted DNA or RNA present in the sample can be positively correlated with the fluorescence of the fluorophore of Probe A." Rather, the '129 patent relies on extended directional non-radiative energy transfer (FRET methodology) for detection of a particular target molecule. The '129 and '387 patents rely on FRET between donor chromophores. In the instant invention there is no FRET methodology involved in the detection of target complementary to SEQ ID NO: 1 (the wanted target). In the instant invention, FRET is only used to increase the performance of SEQ ID NO: 1 by masking the signal of unwanted targets.

The '387 patent does not cure the flaws of the '129 patent. For example, as the Examiner points out, the '387 patent teaches that "when the allele specific probe and the anchor are hybridized the fluorescence is quenched." However, this is in contrast to the current invention, wherein there is no FRET involved in detection of target complementary to SEQ ID NO: 1 (the wanted target). As described above, in the instant invention, FRET is only used to increase the performance of SEQ ID NO: 1 by masking the signal of unwanted targets. Further, the '387 patent nowhere describes the use of a probe which increases the specificity of an assay by preventing signal evolution from the unwanted target.

Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claim 14 stands rejected under 35 U.S.C. 103(a) as being unpatentable over the '129 patent in view of the '387 patent and in further view of Meade et al (US PGPUB 2001/0046679). Applicants respectfully traverse the rejection.

The combination of the '129 and '387 references do not make the subject matter of the instant claims obvious for the reasons stated above. The US PGPUB 2001/0046679 document, like the teaching of the '129 patent, merely provides systems and schemes for probe design, but does not teach or suggest "wherein the presence or amount of wanted DNA or RNA present in the sample can be positively correlated with the fluorescence of the fluorophore of Probe A."

Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claims 2 – 11 and 15 – 20 stand rejected under 35 U.S.C. 103(a) as being unpatentable over the '129 patent in view of the '387 patent et al and in further view of Oliveira et al (Journal of Clinical Microbiology, 2002, Vol. 40, NO. 1, pages 247-251) in view of GenBank S83568 and in further view of Hogan et al (WO0066788). Applicants respectfully traverse the rejection.

The combination of the '129 and '387 references do not make the subject matter of the instant claims obvious for the reasons stated above. Thus, a combination of the '129 patent, the '387 patent and Oliveria does not render the rejected claims obvious. The combination of references fails to teach or suggest , "wherein the presence or amount of wanted DNA or RNA present in the sample can be positively correlated with the fluorescence of the fluorophore of Probe A." The methods of Oliveira are different from those claimed in the instant invention. Further, the methods of Oliviera teach away from the instant invention, showing weak cross-hybridization of the *Staphylococcus aureus* specific probe with *Staphylococcus schleiferi*. Further, Oliveira points out that a *S. aureus* specific probe to this particular region of 16S ribosomal RNA was originally described in a 1993 article (Bentley et. al. Lett. Appl. Microbiol. 16:203-206), and the same probe region was used again in 2000 for FISH experiments (Kempf et. al. J. Clin. Microbiol. 33:50-52). This probe sequence is specific, but it does creates false positive results with *S. schleiferi* despite its

nature as PNA probe and use of design rules by Hogan. Those skilled in the art, including Oliveira, were unable to generate a 100% specific PNA probe (for FISH), despite their efforts, and in addition to the aid of many years of precedent, and the state of the existing technology.

Applicants request withdrawal of the rejection and allowance of the claims.

In view of the above amendment, Applicants believe the pending application is in condition for allowance.

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Respectfully submitted,

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